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INTERACTIVE EFFECTS OF PLANT SPECIES DIVERSITY AND ELEVATED CO_2 ON SOIL BIOTA AND NUTRIENT CYCLING

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Abstract. Terrestrial ecosystems consist of mutually dependent producer and decomposer subsystems, but not much is known on how their interactions are modified by plant diversity and elevated atmospheric CO_2 concentrations. Factorially manipulating grassland plant species diversity and atmospheric CO_2 concentrations for five years, we tested whether high diversity or elevated CO_2 sustain larger or more active soil communities, affect soil aggregation, water dynamics, or nutrient cycling, and whether plant diversity and elevated CO_2 interact. Nitrogen (N) and phosphorus (P) pools, symbiotic N_2 fixation, plant litter quality, soil moisture, soil physical structure, soil nematode, collembola and acari communities, soil microbial biomass and microflora community structure (phospholipid fatty acid [PLFA] profiles), soil enzyme activities, and rates of C fluxes to soils were measured.

No increases in soil C fluxes or the biomass, number, or activity of soil organisms were detected at high plant diversity; soil H_2O and aggregation remained unaltered. Elevated CO_2 affected the ecosystem primarily by improving plant and soil water status by reducing leaf conductance, whereas changes in C cycling appeared to be of subordinate importance. Slowed-down soil drying cycles resulted in lower soil aggregation under elevated CO_2. Collembola benefited from extra soil moisture under elevated CO_2, whereas other faunal groups did not respond.

Diversity effects and interactions with elevated CO_2 may have been absent because soil responses were mainly driven by community-level processes such as rates of organic C input and water use; these drivers were not changed by plant diversity manipulations, possibly because our species diversity gradient did not extend below five species and because functional type composition remained unaltered. Our findings demonstrate that global change can affect soil aggregation, and we advocate that soil aggregation should be considered as a dynamic property that may respond to environmental changes and feed back on other ecosystem functions.

Key words: *biodiversity;* Bromus erectus; *carbon isotopes; $CO_2 \times H_2O$ interactions; global change; ^{15}N isotope dilution; nitrogen and phosphorus; soil aggregation; soil enzymes.*

Introduction

Anthropogenic activities have led to increased atmospheric CO_2 concentrations (Keeling and Whorf 2002) and dramatic losses of species diversity (Pimm et al. 1995), which both affect the functioning of ecosystems. These factors were mostly considered in isolation (e.g., Ewel et al. 1991, Hungate et al. 1997, Hector et al. 1999, Owensby et al. 1999). However, plant diversity and CO_2 enrichment are likely to interact because the CO_2 response of ecosystems depends on the species present and because CO_2 enrichment can also alter plant diversity.

Terrestrial ecosystems consist of mutually dependent producer and decomposer subsystems. While plants provide organic substrate to the predominantly heterotrophic soil community, decomposers affect plant growth by processes such as litter breakdown and mineralization of nutrients. Interactions between plants and soils significantly determine ecosystem function and the diversity of plants and soil communities may be related (Wardle 2002).

Plant diversity affects soil processes and their spatiotemporal heterogeneity in many ways. Patterns of soil exploration by roots depend on species, and the spatiotemporal deposition of organic matter, nutrient acquisition, water fluxes, and so on might therefore depend on plant diversity. Soil community composition has been shown to depend on plant species (De Deyn and van der Putten 2005). Conversely, plants sense rhizosphere conditions and adapt root growth, secretion of secondary compounds, and other processes (Watt et al. 2006). Symbionts and parasites evidently affect plant growth, but many more soil organisms may be involved in interactions less easy to detect. Recent experiments

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indicate that plant growth is not just determined by available nutrients but also by the soil biota present (van der Heijden et al. 1998, De Deyn et al. 2004*a*).

Changes in soil structure are potentially very important aspects of plant–soil interactions, but these have not received much attention in diversity experiments. Biogenic transformations of matter and energy in soil take place in a very heterogeneous physico-chemical environment. Soil structure potentially affects most soil processes and organisms (e.g., pore networks available to fauna, diffusive paths of gases and solutes [Diaz-Zorita et al. 2002]). The processes that determine the spatial organization of pores and soil particles include the binding of mineral particles to microbial products and interactions with roots and hyphae (Tisdall and Oades 1982), and might well depend on plant diversity.

Elevated atmospheric CO_2 interacts with the soil biota in a number of ways, including increased net primary production, alterations of plant community composition due to differences in the ability of species to use this extra resource, and by altered quality of organic matter inputs to soils. For example, increased litterfall and rhizodeposition (Gorissen 1996, Jones and Darrah 1996, Pendall et al. 2004) can affect nutrient availability to plants by immobilization (Diaz et al. 1993) and priming effects (Zak et al. 1996). Symbiotic N_2 fixation often responds positively to the extra availability of carbohydrates under elevated CO_2 (Thomas et al. 2007), altering plant community composition and promoting community productivity. However, not much is known on the role of individual species and species diversity for these responses.

Experimental biodiversity research has mostly focused on plants; linkages between plants and soil biota and soil processes have received less attention. Wardle et al. (1999) created artificial gaps in perennial temperate grassland vegetation and restricted the natural revegetation process to specific combinations of functional groups. During the three-year treatment, microflora, nematodes, collembola, and earthworms were relatively unresponsive to selective removal of single plant functional groups. Within the multi-site BIODEPTH study spanning grassland sites across Europe, effects of plant diversity were found on the soil microbial biomass and earthworms (Spehn et al. 2000); species identity effects were found for collembola (Salamon et al. 2004) and nematodes (Viketoft et al. 2005). Bartelt-Ryser et al. (2005) showed that the growth of plant phytometer species depended on the plant diversity previously grown on soils (soil "memory" effect). One of the only studies containing factorial global change simulations is the BioCON experiment, which comprises species diversity gradients (0–16 perennial herbaceous species) that are factorially combined with elevated CO_2 and N-deposition treatments (Reich et al. 2001). Gross N mineralization increased with plant diversity (Chung et al. 2007). Microbial biomass and activity also increased with diversity and this effect was further enhanced when extra N and CO_2 were supplied in combination (West et al. 2006).

In the present paper, we test interactions between grassland species diversity and atmospheric CO_2 concentrations in a multiyear field study. More specifically, we ask whether high plant diversity or elevated CO_2 (1) sustain larger and/or more active soil communities, (2) lead to differences in soil aggregation, (3) alter nutrient and H_2O dynamics, and (4) whether diversity and CO_2 treatments interact. Our interest in the diversity $\times$ CO_2 interaction bases on the reasoning that not all species in a community might be required for the proper functioning of an ecosystem because of functional redundancy (Walker 1992). This is embodied in the "insurance hypothesis" that states that high diversity communities are less likely to "fail" during "negative" perturbations such as drought (cf. Tilman and Downing 1994, Loreau 2000). Elevated CO_2, providing extra resources, might shift the critical diversity at which an ecosystem function "fails" from higher to lower species diversity. Conversely, high diversity communities might respond more strongly to "positive" perturbations such as CO_2 enrichment because of their higher likelihood to comprise of species that can exploit the extra resources available.

Materials and Methods

Study site and experimental design

The field site is in a nutrient-poor calcareous grassland in northwestern Switzerland (47°33′ N, 7°34′ E; 520 m above sea level; 20° south-facing slope). The native *Mesobromion* community is dominated by *Bromus erectus* Huds. (about one-half of peak-season biomass). *Festuca ovina* and *Festuca rubra* contribute each ~5%; >100 other species account for much lower biomass fractions. The growing period lasts from March to late October. The Rendzina-type soil consists of 10–15 cm top soil (silty clay loam with 30% clay, 56% silt, and 14% sand) underlain by calcareous debris.

Four blocks totaling 36 experimental plots (1.27 m^2) were established in August 1993. The top soil was mixed blockwise before 31, 12, or 5 species per plot were planted in September 1993 (Table 1). All plots contained the matrix-building graminoids *B. erectus* and *F. ovina*. The remaining species were selected so that (1) all communities contained 55% graminoid, 15% legume, and 30% non-legume forb individuals; (2) the most diverse community had approximately the same species richness as the surrounding grassland (31 species per 1 m^2); and (3) the species occurring in the less diverse plant communities were subsets of the species present in the more diverse communities. This resulted in a marked decrease in ranges of plant architectures, growth strategies, and phenologies from high to medium to low diversity, although species selection was in part arbitrary. Effective species richness was 19.3, 7.9, and 3.6 ($e^{H'}$, where H' is the Shannon-Weaver index based on the number of planted individuals); species equitability was identical ($J' = H'/H'_{max} = 0.8$). Most species

were raised from seeds starting in spring 1993. Tillers of the dominant grass *B. erectus* and the subdominant species *F. ovina* and *Trifolium repens* were vegetatively propagated from material collected at the field site.

The plant diversity treatment was factorially combined with an elevated CO_2 treatment that consisted of (1) ambient air control plots (C, 356 μL CO_2/L); (2) ambient-air plots with wind screens (A, 356 μL CO_2/L); and (3) elevated CO_2 plots with wind screens (E, 600 μL CO_2/L). CO_2 enrichment was maintained 24 h/d and shut down only during midwinter (December to February). Overall, the experiment was replicated four times per treatment combination.

Plots were clipped at a height of 5 cm in June and October and the clipped biomass was removed. Mowing or grazing is the common agricultural practice in these grasslands to prevent dominance by rank grass and scrub. Plant diversity treatments were maintained by regular weeding.

Soil sampling and soil physical parameters

Four 3–4 cm diameter × 8 cm depth soil cores were taken from each plot in October 1994, in March, June, and October 1995, in March and June 1996, and in May and June 1998 to determine soil microbial biomass, soil microbial respiration, and soil H_2O. Plots were destructively harvested in June 1998 and large soil blocks (>200 cm^2 surface area, 0–8 cm depth) from the plot's centers used to analyse microbial community structure (phospholipid fatty acid [PLFA] profiles), microfauna, enzyme activities, soil physical structure, and the incorporation of elevated CO_2-C into soil organic matter ($\delta^{13}C$ analysis). At the same time, roots were washed from a large sample (>3000 g; 0–10 cm; see Niklaus et al. 2001*c*) taken from the plot's core area and analysed for lignin and nutrient (N, P) concentrations. Surface litter was collected manually from the entire plot's area and also analysed for lignin and nutrients. Soil H_2O was determined (1) on selected dates by gravimetric determination of H_2O in soil cores (drying for 24 h at 105°C), and (2) continuously by a network of time-domain reflectometry (TDR) probes installed in ambient and elevated CO_2 plots (see Niklaus et al. [1998*b*] for sensor and multiplexer design).

Soil particle size/density fractions were determined as described in Niklaus et al. (2001a). Briefly, soil samples were suspended in 0.5% sodium hexametaphosphate, washed through a stack of sieves (2000, 1000, 500, 250, and 125 μm) and the size fractions obtained separated by flotation on sodium polytungstate solutions (SOMETU, Falkenried, Germany) with a density of 1.7 g/cm^3. The organic fraction >2000 μm was discarded because it contained live roots rather than particulate macroorganic matter.

Soil biota

Soil basal respiration and substrate-induced respiration (SIR) were measured in sieved (2 mm) fresh soil preincubated at 22°C for three days at 50% water holding capacity (Niklaus 1998). The metabolic quotient for CO_2 (qCO_2, h^{-1}) was obtained by dividing basal respiration by the microbial biomass calculated from SIR (Anderson and Domsch 1978). Soil microbial C and N were also determined by chloroform fumigation-extraction (Brookes et al. 1985, Vance et al. 1987).

The activities of soil enzymes (urease, protease, arginine deaminase, trehalase, xylanase, invertase, arylsulphatase, alkaline phosphomonoesterase) were determined in laboratory incubations with the respective substrates (protocols in Schinner et al. [1996] and Ebersberger et al. [2003]).

PLFA were measured following Bardgett et al. (1996) and Frostegård et al. (1993). Fatty acid methyl esters were analysed by gas chromatography–mass spectrometry (HP-5 capillary column; Agilent, Palo Alto, California, USA) and methyl esters assigned to PLFAs using Supelco standards (Sigma-Aldrich, Taufkirchen, Germany). Bacterial biomass was estimated from nine bacterial PLFA (i15:0, a15:0, 15:0, i16:0, i17:0, 17:0,

TABLE 1. Plant species composition (%) in the diversity treatments.

Functional type and species	Plant species diversity		
	High	Medium	Low
Grasses			
Bromus erectus	30.5	30.5	55.0
Festuca ovina	10.0	24.5	
Briza media	2.4		
Koeleria pyramidata	2.4		
Dactylis glomerata	2.4		
Cynosurus cristatus	2.4		
Anthoxanthum odoratum	2.4		
Carex flacca	2.4		
Legumes			
Lotus corniculatus	2.7	6.7	7.9
Trifolium montanum	2.7	7.0	7.9
Trifolium repens	2.1	2.1	
Trifolium pratense	2.7		
Anthyllis vulneraria	2.7		
Medicago lupulina	2.7		
Non-legume forbs			
Prunella vulgaris	4.5	4.5	14.5
Prunella grandiflora	4.5	4.5	14.8
Salvia pratensis	2.1	5.1	
Hieracium pilosella	2.1	5.1	
Bellis perennis	1.8	5.1	
Plantago media	1.2	2.4	
Plantago lanceolata	1.2	2.4	
Gentianella germanica	3.6		
Gentiana cruciata	0.9		
Knautia arvensis	0.9		
Scabiosa columbaria	0.9		
Centaurea jacea	0.9		
Sanguisorba minor	0.9		
Ranunculus bulbosus	0.9		
Campanula glomerata	0.9		
Pimpinella saxifraga	0.9		
Leucanthemum vulgare	0.9		

Notes: Numbers given are percentages of planted individuals in the respective treatment. All species were naturally growing at the site.

cy17:0, 18:1ω7, cy19:0) while 18:2ω6,9 served as fungal marker (Frostegård and Bååth 1996). Ergosterol was extracted from soils with ethanol, dried at 40°C in a rotary evaporator and the residue re-dissolved in 5 mL ethanol. Ergosterol was separated by RP-HPLC and determined at 282 nm (Djajakirana et al. 1996).

Soil microarthropods were collected from a 90 $cm^2 \times$ 8 cm deep soil block using a Kempson extractor. Soil surface temperature was increased from 25°C to 50°C over one week, animals collected in picric acid, stored in 70% ethanol, and counted. Nematodes were fixed in soil with 5% formalin at 60°C, extracted using an Oostenbrink elutriator (Oostenbrink 1960), cleaned using a centrifugal flotation technique ($MgSO_4$(aq) with $\rho = 1.2$ g/cm^3, 18 000 m/s^2, 4 min.; cf. Southey 1986) and counted under a dissection microscope.

Soil carbon cycling

In order to test for treatment effects on C cycling, total soil C and N, extractable soil C and N, root and surface litter lignin concentration as important indicator of recalcitrance, and the net incorporation of C fixed during the experiment into soils (using an isotopic tracer) were measured at termination of the experiment.

Total soil organic C and N were determined by dry combustion (automated C-H-N analyzer, LECO CHN-1000; LECO Corporation, St. Joseph, Michigan, USA). Samples were prepared by sieving (500 μm mesh size) and grinding. Amounts of dissoluble organic C and N were determined by extracting soil samples (30 g dry mass) with 100 mL 0.5 mol/L K_2SO_4 and with 60 mL hot demineralized water (60°C). Dissolved organic C and N in these extracts were determined with a TOC/TN_b analyzer (DIMA TOC-100; Dimatec, Essen, Germany). Lignin in roots and litter was extracted with thioglycol (method by Bruce and West [1989]) and extracted lignin determined as described in Hirschel et al. (1997). Finally, net incorporation of C fixed under elevated CO_2 could be calculated because the commercial CO_2 used for atmospheric enrichment was depleted in ^{13}C ($\delta^{13}C \approx -35‰$) relative to atmospheric CO_2 ($\delta^{13}C = -8‰$). Based on $\delta^{13}C$ measurements of plant and bulk soil material from ambient and elevated CO_2 (Finnigan MAT delta S IRMS; Finnigan MAT, Bremen, Germany), soil C was partitioned into pre-experimental and elevated CO_2-derived C and diversity treatments compared (for details of the method, see Niklaus et al. [2001*d*]).

Nitrogen and phosphorus relations

Symbiotic N_2 fixation was determined by ^{15}N isotope dilution. Plots had been amended with $^{15}NH^{15}NO_3$ on 14 March, 29 April, and 30 June 1995 (52 mg $^{15}N/m^2$ in total). We determined δ15N in shoots of legume and non-legume material collected in June 1995, October 1995, June 1996, and October 1996 (Finnigan MAT delta S IRMS; Finnigan MAT, Bremen, Germany). The fraction of legume N originating from symbiotic fixation was calculated as $\%N_{dfa} = (^{15}N_{nonl} - {}^{15}N_{leg})/(^{15}N_{nonl} - {}^{15}N_{atm})$, where $^{15}N_{leg}$, $^{15}N_{nonl}$, and $^{15}N_{atm}$ are the ^{15}N mole fractions of N in legumes, all non-legume plants present in the plot, and atmospheric N_2, respectively (Wood and McNeill 1993). Plant N and P pools were determined by dry combustion (CHN-900, LECO Instruments) and by a micro-Kjehldahl-type wet digestion and colorimetric determination of PO_4^{3-} (Niklaus et al. 1998*a*).

Statistical analysis

Data were analyzed by analysis of variance. All data were first log-transformed to meet the assumption of normal distribution. All model terms were fitted sequentially (type I sum of squares). Factors were block, plant diversity (div), CO_2 treatment (CO_2), and the div × CO_2 interaction. Repeated-measures analysis of variance was used to test for effects of time and interactions of time with treatments. For analysis of data below the plot level (aggregate size distribution), a split-plot model was used which contained the sub-plot terms size, div × size, CO_2 × size, and div × CO_2 × size.

Results

Soil moisture and soil physical structure

Soil H_2O exhibited pronounced temporal variability (TDR measurements, data not shown). Plant diversity had no effect on soil H_2O (TDR measurements and gravimetric determinations; Fig. 1). Elevated CO_2 significantly increased soil H_2O, which compensated a drying effect of the installed wind screens. Though highly significant, these effects were minor compared to the seasonal and interannual variability in soil H_2O.

After physical fractionation, most soil was recovered as high-density organo-mineral aggregates (Fig. 2). Plant diversity had no effect but elevated CO_2 reduced aggregate sizes ($F_{4,72} = 5.11$, $P < 0.01$ for size × CO_2); this effect was also found for the amount of C contained in these fractions ($F_{4,72} = 4.80$, $P < 0.01$). No other treatment effects were found (CO_2, diversity, CO_2 × diversity, diversity × size, and CO_2 × diversity × size all were not significant).

The low-density macro-organic fraction was not affected by experimental treatments.

Soil organic C and N

Soil organic C (0.321 ± 0.003%) and N (0.0280 ± 0.0004%), 0.5 mol/L K_2SO_4-extractable C (67 ± 3 μg C/g soil) and N (14 ± 1 μg N/g soil), and hot-water extractable organic C (142 ± 13 μg C/g soil) and N (11 ± 1 μg N/g soil) all were unaffected by experimental treatments (all values are means ± SE).

We found that 469 ± 27 g C/m^2 fixed under the five years of CO_2 exposure (C_{new}) had been incorporated into soils (excluding the macro-organic fraction >2000 μm, which contained mostly root fragments). Amounts of C_{new} did not depend on plant diversity. Most C_{new} was stored in high-density organo-mineral aggregates (331 ± 20 g C_{new}/m^2) although these contained ≈90%

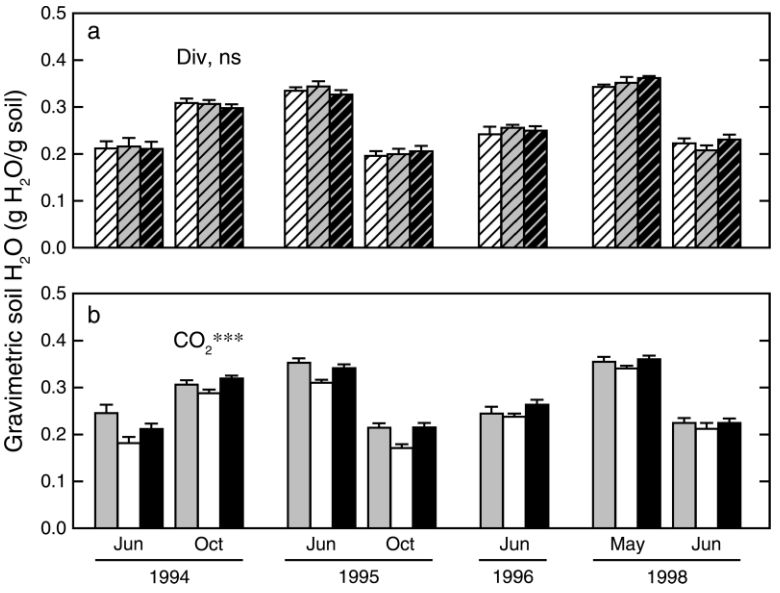

FIG. 1. Soil H_2O content (gravimetrically determined), showing effects of (a) plant diversity (white, low; gray, medium; black, high species diversity) and (b) elevated CO_2 treatments (gray, unscreened control plots; white, screened ambient CO_2 plots; black, screened elevated CO_2 plots). Bars indicate means + SE.

*** $P < 0.001$; ns, not significant.

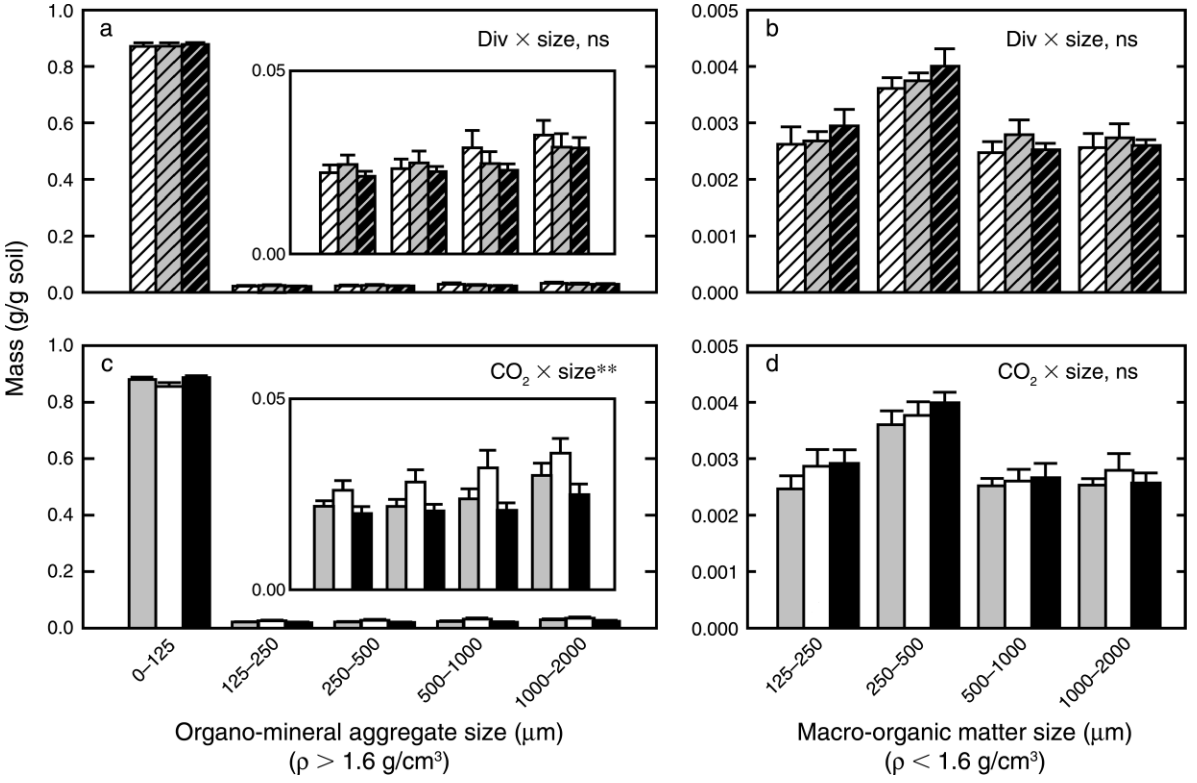

FIG. 2. Soil particle size and density (ρ) fractions as function of (a and b) plant diversity (white, low; gray, medium; black, high plant species diversity) and (c and d) windscreen/CO_2 treatment (gray, unscreened control plots; white, screened ambient CO_2 plots; black, screened elevated CO_2 plots). Plant diversity did not affect (a) high-density organo-mineral aggregates or (b) low-density macro-organic matter. In contrast, elevated CO_2 induced a shift in (c) size distribution of organo-mineral aggregates but did not affect (d) low-density macro-organic fractions. Bars indicate means + SE.

** $P < 0.01$; ns, not significant.

TABLE 2. Soil enzyme activities in June 1998 (peak biomass).

Enzyme or activity	Units	Treatment					
		Low diversity			Medium diversity		
		C	A	E	C	A	E
Xylanase	μg GE·g soil^{-1}·d^{-1}	1857 ± 151	1894 ± 108	1819 ± 156	1957 ± 107	1789 ± 133	1698 ± 117
Invertase	μg GE·g soil^{-1}·3 h^{-1}	4544 ± 256	5005 ± 319	5370 ± 138	5462 ± 220	4987 ± 191	4945 ± 242
Trehalase	μg GE·g soil^{-1}·3 h^{-1}	864 ± 33	916 ± 53	863 ± 51	1089 ± 90	1031 ± 11	864 ± 36
Urease	μg NH_4^+-N·g soil^{-1}·2 h^{-1}	36.5 ± 1.3	46.7 ± 3.3	56.5 ± 2.8	53.2 ± 3.2	45.2 ± 2.4	48.3 ± 3.0
Deaminase	μg NH_4^+-N·g soil^{-1}·2 h^{-1}	12.5 ± 0.4	12.0 ± 0.3	11.5 ± 0.2	12.0 ± 0.6	10.8 ± 0.3	11.2 ± 0.4
Protease	μg N·g soil^{-1}·3 h^{-1}	505 ± 31	398 ± 21	499 ± 15	465 ± 7	374 ± 31	426 ± 30
Phosphatase	μg phenol·g soil^{-1}·3 h^{-1}	1373 ± 75	1367 ± 110	1462 ± 52	1370 ± 51	1259 ± 95	1311 ± 64
Arylsulphatase	μg nitrophenol·g soil^{-1}·h^{-1}	139 ± 14	138 ± 4	158 ± 3	178 ± 10	146 ± 17	136 ± 4
N-mineralization§	μg N·g soil^{-1}·d^{-1}	46 ± 4	42 ± 5	46 ± 2	58 ± 8	51 ± 4	46 ± 4
Nitrification§	μg N·g soil^{-1}·5 h^{-1}	296 ± 25	481 ± 89	411 ± 31	295 ± 36	365 ± 57	283 ± 39

Notes: Nitrogen mineralization and nitrification rates are included for reference (see Niklaus et al. 2001*b*); values are means ± SE. "GE" indicates glucose equivalents. CO_2 treatments: C, unscreened control plots; A, screened ambient CO_2 plots; E, screened elevated CO_2 plots.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

† Diversity.

‡ This column reports the effects of windscreen alone/effect of elevated CO_2 within windscreen. In the presence of a Div × CO_2 interaction due to both windscreen and elevated CO_2, only the combined test for windscreen/CO_2 is given (factor with three levels).

§ Data from Niklaus et al. (2001*b*) included for reference.

C_{old}. Following the aggregate mass distribution, most of this C_{new} was bound to the size fraction <125 μm. The low-density macro-organic fraction was labeled to a larger extent than the organo-mineral fraction (≈50%) but, due to its small size, this pool only contained 138 ± 11 g C_{new}/m^2.

Root C:N, N:P, lignin concentration, and lignin:N were not affected by treatments (38.8 ± 0.9 g C/g N, 16.8 ± 0.3 g N/g P, 10.1 ± 0.3%, and 11.6 ± 0.2 g lignin/g N, respectively). The quality of surface litter collected at the end of the experiment did not depend on diversity treatments but elevated CO_2 increased C:N ($P = 0.01$), lignin:N ($P < 0.05$) and lignin:P ($P = 0.01$) compared to ambient conditions; lignin concentrations did not change significantly (C:N = 51 ± 2 and 57 ± 2 g C/g N; lignin:N = 10.6 ± 0.7 and 12.2 ± 0.6 g lignin/g N; lignin:P = 192 ± 7 and 224 ± 16 g lignin/g P; lignin = 8.7 ± 0.5 and 8.2 ± 0.3% in ambient and elevated CO_2, respectively).

Soil biota

Soil microbial biomass varied around ≈1000 μg C/g soil and the metabolic quotient for CO_2 (qCO_2) averaged at 1.7 × 10^{-3} h^{-1} (range of 1.1–2.4 × 10^{-3} h^{-1}), depending on year and season. Neither measure depended on treatments.

TABLE 3. Soil microfauna individual numbers as function of plant diversity and atmospheric CO_2 treatment.

Community	Treatment					
	Low diversity			Medium diversity		
	C	A	E	C	A	E
Microarthropods§						
Total	45.7 ± 9.6	62.9 ± 13.7	80.3 ± 21.1	70.9 ± 10.0	69.3 ± 21.2	77.3 ± 10.1
Acari	25.2 ± 6.2	41.7 ± 10.1	49.7 ± 11.0	45.7 ± 10.5	53.2 ± 19.2	50.4 ± 11.2
Collembola	7.1 ± 1.4	3.2 ± 0.8	9.1 ± 1.9	7.4 ± 1.1	5.8 ± 2.4	8.3 ± 1.0
Symphypleona	2.3 ± 0.7	0.5 ± 0.3	1.4 ± 0.2	1.3 ± 0.9	0.8 ± 0.6	1.3 ± 0.7
Entomobryidae	2.0 ± 0.8	0.7 ± 0.3	3.4 ± 1.5	2.1 ± 0.1	2.1 ± 1.3	4.1 ± 0.9
Onychiuridae	2.6 ± 0.6	2.0 ± 0.7	5.2 ± 1.7	3.1 ± 0.9	2.5 ± 1.3	2.8 ± 0.6
Protura	1.9 ± 0.6	5.8 ± 2.4	4.3 ± 3.1	3.4 ± 0.9	1.4 ± 1.1	3.6 ± 1.6
Diplura	1.9 ± 0.6	5.8 ± 2.4	4.3 ± 3.1	3.4 ± 0.9	1.4 ± 1.1	3.6 ± 1.6
Pauropoda	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.7 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Symphyla	0.8 ± 0.2	0.5 ± 0.1	0.7 ± 0.2	0.6 ± 0.2	0.2 ± 0.1	0.9 ± 0.3
Nematodes¶	0.74 ± 0.10	0.80 ± 0.17	0.51 ± 0.09	1.28 ± 0.53	1.19 ± 0.25	1.83 ± 1.11

Notes: Values are means ± SE. CO_2 treatments: C, unscreened control plots; A, screened ambient CO_2 plots; E, screened elevated CO_2 plots.

* $P < 0.05$; ** $P < 0.01$.

† Diversity.

‡ This column reports the effects of windscreen alone/effect of elevated CO_2 within windscreen. In the presence of a Div × CO_2 interaction due to both windscreen and elevated CO_2, only the combined test for windscreen/CO_2 is given (factor with three levels).

§ All values multiplied by 10^3.

¶ All values multiplied by 10^6.

TABLE 2. Extended.

Treatment			ANOVA		
High diversity					
C	A	E	Div†	CO_2‡	Div × CO_2
1579 ± 54	1892 ± 147	1553 ± 99	ns	ns	ns
4849 ± 73	5064 ± 266	5337 ± 326	ns	ns	ns
1025 ± 16	1316 ± 230	977 ± 82	**	ns/*	ns
47.6 ± 1.5	46.8 ± 1.3	49.5 ± 0.3	ns	**	***
11.0 ± 0.3	11.3 ± 0.3	12.1 ± 0.7	ns	ns	ns
564 ± 37	494 ± 31	541 ± 22	***	**/***	ns
1279 ± 48	1313 ± 56	1207 ± 85	ns	ns	ns
132 ± 12	136 ± 9	146 ± 5	ns	ns	**
58 ± 5	59 ± 10	59 ± 4	ns	ns	ns
174 ± 45	267 ± 54	184 ± 64	***	*/*	ns

Trehalase and protease activities increased at high diversity (Table 2). Elevated CO_2 increased protease (+16%, $P < 0.01$) and decreased trehalase (−17%, $P < 0.05$) activities, while windscreens had opposite effects approximately equal in magnitude (protease, −17%, $P < 0.01$; trehalase, +10%, not significant). Arylsulphatase and urease revealed significant diversity × CO_2 interactions, but these responses were seemingly idiosyncratic.

Individual PLFA (see Appendix) did not respond to treatments except for 10Me17:0, which showed a significant positive effect of windscreen and a negative effect of CO_2 enrichment, and for a diversity-effect for 15:0 (but this may be a type I error given the large number of tests and the weak significance). PLFA did not indicate a shift in bacteria : fungi; ergosterol concentration, an indicator of fungal biomass, also remained unaffected by plant diversity and elevated CO_2.

TABLE 3. Extended.

Treatment			ANOVA		
High diversity					
C	A	E	Div†	CO_2‡	Div × CO_2
77.0 ± 17.0	71.2 ± 4.2	49.1 ± 10.4	ns	ns	ns
42.0 ± 14.1	43.5 ± 10.4	24.2 ± 7.3	ns	ns	ns
14.8 ± 3.2	5.1 ± 1.4	8.5 ± 2.3	ns	**/**	ns
3.9 ± 2.0	1.3 ± 0.6	1.9 ± 0.9	ns	*/*	ns
6.0 ± 2.9	1.2 ± 0.1	2.2 ± 0.9	ns	**/**	ns
4.9 ± 0.8	2.5 ± 1.0	4.2 ± 1.5	ns	**/**	ns
1.7 ± 0.5	7.6 ± 4.1	2.6 ± 0.8	ns	**/**	ns
1.7 ± 0.5	7.6 ± 4.1	2.6 ± 0.8	ns	ns	ns
0.1 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	ns	ns	ns
0.1 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	*	ns	ns
1.70 ± 0.19	1.13 ± 0.23	1.20 ± 0.30	ns	ns	ns

Total microarthropod numbers equaled $67 \pm 4 \times 10^3$ individuals/m^2 (Table 3) and were independent of plant diversity and elevated CO_2. Acari also showed no treatment effects ($42 \pm 4 \times 10^3$ individuals/m^2), but collembola numbers increased by 83% ($P < 0.01$) under elevated CO_2 and decreased in the presence of wind screens (−52%, $P < 0.01$; Table 3). This order-level effect was due to similar significant effects within the *Symphypleona* sub-order and the *Entomobryidae* and *Onychiuridae* families. The other identified orders (*Protura*, *Diplura*, *Pauropoda*, *Symphyla*) did not significantly depend on treatments. Total nematode numbers ($1.15 \pm 0.15 \times 10^6$ individuals/m^2; Table 3) did not depend on treatments (note that this does not include root residing nematodes due to the extraction method used).

Nitrogen and phosphorus cycling

Legumes derived virtually all N from the atmosphere ($\%N_{dfa} \approx 90\%$), independent of treatments. Over the whole experiment, 26% more N was recovered in clipped shoots in high (19.9 ± 0.8 g N/m^2) compared to low or medium diversity (15.4 ± 1.0 and 16.1 ± 1.0 g N/m^2). No diversity effects were found for root N (12.5 ± 0.6 g N/m^2). At the whole-plant level, there was only a marginally significant increase at high diversity (+16%, $P = 0.06$). Elevated CO_2 affected neither shoot nor root N. Plant P content was only determined in June of 1995, 1996, and 1998 so that no complete P balance could be calculated; however, no treatment effects were found on these dates, suggesting similar (no) responses for N and P.

DISCUSSION

Despite five and a half years of plant diversity manipulation, relatively few effects on soil biota and processes were found. In contrast to our hypotheses, no increases in soil C fluxes or the biomass, number, or activity of soil organisms sustained at high plant diversity were detected. Soil H_2O and aggregation also remained unaltered by plant species diversity. Notable exceptions to the general absence of diversity effects were increases in the activities of two soil enzymes (trehalase and protease) at high plant diversity. Furthermore, the activity of earthworms increased with plant species numbers (previously reported by Zaller and Arnone [1999*a*]) and soil nitrate concentrations and nitrification rates were reduced at high diversity (Niklaus et al. 2001*b*; Table 2). Elevated CO_2 did not sustain higher soil microbial biomass but collembola numbers increased. Among the most important effects of CO_2 enrichment were increases in soil H_2O and decreases in soil aggregation. Unlike hypothesized, the elevated CO_2 treatment did not interact with plant diversity with respect to most parameters.

Relatively few studies have addressed plant diversity effects on soil consumers other than the microflora and these indicate that effects can depend on trophic position: For example, Wardle et al. (2004) manipulated

aphid diversity in model microcosms and this induced shifts in plant community composition which in turn changed the abundance of belowground secondary (but not primary and tertiary) consumers. De Deyn et al. (2004*b*) reported that both plant species identity and diversity affected nematode community composition. Despite these uncertainties, plant organic C inputs to soils certainly are a major factor limiting at least parts of belowground communities (Wardle 2002). Plant diversity positively correlated with primary production in many studies (Hooper et al. 2005), suggesting better supply of soils with C at high diversity. In our study, aboveground primary production increased at high species diversity, but only when communities were exposed to elevated CO_2, and only on some dates (Niklaus et al. 2001*c*). The data presented here do not indicate that this translated into more than possibly a small increase in soil C inputs. Extractable organic C and soil macro-organic matter did not indicate increased availability of organic C, and most importantly, net fluxes of recently fixed C into soils did not show effects of plant diversity. Neither did the soil microbial biomass respond to plant diversity nor were compositional changes detected in PLFA profiles, but Grüter et al. (2006) found that plant diversity altered bacterial species composition (but not richness; OTUs defined by T-RFLP of 16S rDNA). Nematodes, acari and collembola abundances did not show any effect of plant diversity treatments. The only faunal group responding to diversity was lumbricid earthworms, an effect most likely related to the association of earthworm activity with specific plant species (Zaller and Arnone 1999*b*). Positive effects of plant diversity on earthworms were also reported from the BIODEPTH experiment (Spehn et al. 2000).

Soil fauna can alter soil structure by ingesting and excreting soil and organic matter, by litter comminution, and by creating soil pores and burrows (Lee and Foster 1991). Earthworms have a particularly important role due to the large amount of soil they process and the profound changes in soil structure that are associated with cast formation (Oades 1993, Pulleman et al. 2005, Bossuyt et al. 2006). Blanchart et al. (1997) demonstrated that aggregation of savannah soil was promoted by earthworms and that the size of aggregates formed was species specific. However, increased earthworm activity did not change soil structure in our study, possibly because earthworm effects are less important in clay soils such as the present (Schrader and Zhang 1997). In high clay soils, soil shrinkage during drying cycles is a dominant driver of aggregate formation (Oades 1984). Plants can affect evapotranspiration and therefore drying cycles at the ecosystem scale; at a smaller scale, local drying of soil by plant roots hardens aggregates more than any other biotic process (Young et al. 1998). However, soil H_2O dynamics were not affected by plant diversity manipulations in the present study. A further way in which plant diversity could have altered soil structure (but did not) is through interspecific differences in root architecture and spatiotemporal patterns of rhizodepositions; organic matter deposited in soils acts as a binding agent in aggregates, and roots and fungal hyphae can link and encage soil particles but also break soil structures by exercising radial pressure (Oades 1993).

Community productivity of the grassland studied is N limited (Niklaus and Körner 2004). High diversity communities captured more soil mineral N and this resulted in larger amounts of N harvested in clipped shoots plus roots. In contrast, low-diversity communities were less efficient in capturing soil NH_4^+, which manifested in increased nitrification and soil NO_3^- concentrations (Niklaus et al. 2001*b*). Laboratory incubations showed no effect on N mineralization rates, but these were measured at optimal soil H_2O, whereas H_2O was often limiting in the field. It therefore remains unclear whether increased plant N at high diversity was the result of higher mineralization or of more efficient capture of potentially available soil mineral N. Regardless of the mechanism involved, the increase in plant N and biomass was small compared to other field studies (Hooper et al. 2005); our results also contrast with Chung et al. (2007) and West et al. (2006) who reported increased N mineralization and microbial biomass and activity at high species diversity. The largest drops in productivity were generally found when species numbers fell below five or when functional type composition was reduced (cf. Tilman et al. 1996, Hooper and Vitousek 1997, Hector et al. 1999). For example, Spehn et al. (2002) showed that legume presence was a main determinant of increased productivity at high diversity in the BIODEPTH experiment. However, this lower end of the plant diversity gradient was not part of our design since we kept functional type composition constant and retained the matrix forming species together with some subdominants in all plots.

Elevated CO_2 profoundly altered water dynamics; soils in screened ambient plots were generally drier than in unscreened control plots. This effect was fully compensated by reduced stomatal conductance and resulting lower evapotranspiration under CO_2 enrichment. While these soil H_2O effects were relevant for ecosystem functioning, they did not significantly affect site climate: seasonal and interannual variability in soil H_2O was larger by orders of magnitude (Niklaus et al. 2001*c*). Elevated CO_2 also altered soil aggregation. We argue that the changes in aggregate structure were driven by the soil H_2O effects observed, for several reasons. First, effects on aggregate sizes exactly mirror the changes found for soil H_2O. Second, soil shrinkage during drying cycles is a main driver of aggregate formation (Oades 1984). Third, while direct effects of elevated CO_2 could affect aggregation by increasing fine root turnover, mucilage production, and mycorrhizal hyphal growth (Oades 1984), these changes should result in increased aggregation instead of a decrease; furthermore, this mechanism would not explain the opposite

effects of screening. We have detected similar effects on soil structure after six years of CO_2 treatment in a second study conducted on the same site in plots with undisturbed vegetation and soils (Niklaus et al. 2003), and after one year in a third study that utilized intact soil monoliths that were transferred to the greenhouse (Niklaus et al. 2001*a*). All three observations were made in different years so that effects were not related to the weather conditions in a particular year. We are therefore confident that indirect, H_2O-driven effects of CO_2 on soil aggregation are a general phenomenon, at least for this type of soil and vegetation. Rillig et al. (1999), studying a Mediterranean annual grassland, reported that elevated CO_2 promoted aggregation and argued that this was related to increased production of glomalin in elevated CO_2 plots (Rillig et al. 1999). Preliminary measurement in undisturbed plots at the site of the present study, however, show that glomalin concentrations were also increased by elevated CO_2 (P. A. Niklaus and M. C. Rillig, *unpublished data*), indicating that mechanisms depend on the specific site conditions. We hypothesize that at our site aggregation was predominantly driven by soil H_2O dynamics due to its high soil clay content, while biotic processes may be more important at sites with coarser textured soils.

Soil biota responses to CO_2 enrichment were equivocal: No effects were found for the soil microflora except for a decrease of a single PLFA (10Me17:0) which is indicative of *Actinomycetes* and probably related to soil H_2O changes (Zelles 1999). T-RFLP analysis (Grüter et al. 2006) also did not reveal CO_2 effects at the level of the OTUs resolved. Nematode and acari abundances were unresponsive, but collembola and lumbricid earthworms responded positively to elevated CO_2 and negatively to screening of plots. We hypothesize that these effects were again driven by soil H_2O changes, for two reasons: First, response patterns closely followed the soil H_2O effects. Second, earthworm and collembola (that dwell in inter-aggregate voids) probably respond more strongly to desiccation than nematodes (that inhabit water films on aggregate surfaces). Arguments about differential responses of trophic levels conventionally center around limitation by resources versus predation (Hairston et al. 1960, Wardle 2002); our observations raise the interesting possibility that indirect effects via environmental changes might be as important. However, we acknowledge that the evidence presented here is not conclusive; e.g., increased hyphal growth at increased soil H_2O could produce similar patterns under bottom-up limitation.

Plant and soil microbial N pools did not respond to elevated CO_2, but soil NO_3^- concentrations were reduced and potential nitrification rates increased. Mineralization rates showed no effects of CO_2 enrichment, which is in-line with many other studies (e.g., West et al. 2006 and compilation of studies in Niklaus 2007). CO_2 effects on protease activities are most likely driven by soil moisture changes (protease activities generally drop under desiccation). Symbiotic N_2 fixation is energy-demanding, and legumes therefore often benefit more from CO_2 enrichment than non-N_2-fixing species (e.g., Zanetti et al. 1996). This mechanisms can relieve communities from N limitation and result in higher plant productivity. However, in the present study, the growth of legumes was limited by available phosphorus so that amounts of atmospheric N_2 introduced into biotic pools did not increase under elevated CO_2. Elevated CO_2 increased the lignin:N ratio of surface litter collected at the end of the study. This might indicate decreased decomposability of plant inputs under elevated CO_2, but it is also possible that the more easily decomposable fractions had already been decomposed under elevated CO_2 (e.g., due to higher soil H_2O), leaving back a larger fraction of the more recalcitrant material.

Conclusions

Long-term field studies experimentally addressing linkages between plant and soil diversity are scarce, despite many indications that diversity and functioning of plant and belowground communities are intimately intertwined. Plant diversity did not affect most parameters in the present study (notable exceptions are soil NO_3^- and earthworm activity), and there were virtually no interactions with elevated CO_2. This is surprising both in the light of potential mechanisms and other studies. At least three reasons account for the absence of effects. First, the functional redundancy of soil species may be quite large due to the high species diversity in soils. Second, it is possible that our diversity treatments had effects on community structure at a taxonomic level that we have not resolved with our experimental protocols. Third, at the relatively coarse taxonomic level that we have resolved, effects on soils might be driven mainly by community-level processes such as overall water use or rates of organic matter input to soils; however, our plant diversity treatments did only have small effects on community productivity and functional type composition was held constant.

Elevated CO_2 affected belowground communities (but did not interact with plant diversity). It appears that the main driver was improved plant and soil water status due to reduced leaf conductance of plants. An important finding was that altered drying cycles reduced soil aggregation. Soil structure currently receives only limited attention in global change research; we advocate that aggregation should be considered as a dynamic property that may respond to environmental changes, and that feedback via aggregation may be important for some ecosystem functions (e.g., C storage).

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Literature Cited

Anderson, J. P. E., and K. H. Domsch. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry 10:215–221.

Bardgett, R. D., P. J. Hobbs, and A. Frostegård. 1996. Changes in soil fungal : bacterial biomass ratios following reductions in the intensity of management of an upland grassland. Biology and Fertility of Soils 22:261–264.

Bartelt-Ryser, J., J. Joshi, B. Schmid, H. Brandl, and T. Balser. 2005. Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. Perspectives in Plant Ecology, Evolution and Systematics 7:27–49.

Blanchart, E., P. Lavelle, E. Braudeau, Y. LeBissonnais, and C. Valentin. 1997. Regulation of soil structure by geophagous earthworm activities in humid savannas of Cote d'Ivoire. Soil Biology and Biochemistry 29:431–439.

Bossuyt, H., J. Six, and P. F. Hendrix. 2006. Interactive effects of functionally different earthworm species on aggregation and incorporation and decomposition of newly added residue carbon. Geoderma 130:14–25.

Brookes, P. C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17: 837–842.

Bruce, R., and C. A. West. 1989. Elicitation of lignin biosynthesis and isoperoxidase activity by pectic fragments in suspension cultures of castor bean. Plant Physiology 91: 889–897.

Chung, H., D. R. Zak, P. B. Reich, and D. S. Ellsworth. 2007. Plant species richness, elevated CO_2, and atmospheric nitrogen deposition alter soil microbial community composition and function. Global Change Biology 13:980–989.

De Deyn, G. B., C. E. Raaijmakers, and W. H. van der Putten. 2004*a*. Plant community development is affected by nutrients and soil biota. Journal of Ecology 92:824–834.

De Deyn, G. B., C. E. Raaijmakers, J. van Ruijven, F. Berendse, and W. H. van der Putten. 2004*b*. Plant species identity and diversity effects on different trophic levels of nematodes in the soil food web. Oikos 106:576–586.

De Deyn, G. B., and W. H. van der Putten. 2005. Linking aboveground and belowground diversity. Trends in Ecology and Evolution 20:625–633.

Diaz, S., J. P. Grime, J. Harris, and E. McPherson. 1993. Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. Nature 364:616–617.

Diaz-Zorita, M., E. Perfect, and J. H. Grove. 2002. Disruptive methods for assessing soil structure. Soil and Tillage Research 64:3–22.

Djajakirana, G., R. G. Joergensen, and B. Meyer. 1996. Ergosterol and microbial biomass relationship in soil. Biology and Fertility of Soils 22:299–304.

Ebersberger, D., P. A. Niklaus, and E. Kandeler. 2003. Long term CO_2 enrichment stimulates N-mineralisation and enzyme activities in calcareous grassland. Soil Biology and Biochemistry 35:965–972.

Ewel, J. J., M. J. Mazzarino, and C. W. Berish. 1991. Tropical soil fertility changes under monoculture and successional communities of different structure. Ecological Applications 1:289–302.

Frostegård, A., and E. Bååth. 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22:59–65.

Frostegård, A., E. Bååth, and A. Tunlid. 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty-acid analysis. Soil Biology and Biochemistry 25:723–730.

Gorissen, A. 1996. Elevated CO_2 evokes quantitative and qualitative changes in carbon dynamics in a plant/soil system: mechanisms and implications. Plant and Soil 187:289–298.

Grüter, D., B. Schmid, and H. Brandl. 2006. Influence of plant diversity and elevated atmospheric carbon dioxide levels on belowground bacterial diversity. BMC Microbiology 6: Article 68.

Hairston, N. G., F. E. Smith, and L. B. Slobodkin. 1960. Community structure, population control, and competition. American Naturalist 94:421–425.

Hector, A., et al. 1999. Plant diversity and productivity experiments in European grasslands. Science 286:1123–1127.

Hirschel, G., C. Körner, and J. A. Arnone. 1997. Will rising atmospheric CO_2 affect leaf litter quality and in situ decomposition rates in native plant communities? Oecologia 110:387–392.

Hooper, D. U., et al. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75:3–35.

Hooper, D. U., and P. M. Vitousek. 1997. The effects of plant composition and diversity on ecosystem processes. Science 277:1302–1305.

Hungate, B. A., E. A. Holland, R. B. Jackson, F. S. Chapin, H. A. Mooney, and C. B. Field. 1997. The fate of carbon in grasslands under carbon dioxide enrichment. Nature 388: 576–579.

Jones, D. L., and P. R. Darrah. 1996. Re-sorption of organic compounds by roots of *Zea mays* L. and its consequences in the rhizosphere. Plant and Soil 178:153–160.

Keeling, C., and T. P. Whorf. 2002. Atmospheric CO_2 records from sites in the SIO air sampling network. *In* Trends: a compendium of data on global change. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee, USA.

Lee, K. E., and R. C. Foster. 1991. Soil fauna and soil structure. Australian Journal of Soil Research 29:745–775.

Loreau, M. 2000. Biodiversity and ecosystem functioning: recent theoretical advances. Oikos 91:3–17.

Niklaus, P. A. 1998. Effects of elevated atmospheric CO_2 on soil microbiota in calcareous grassland. Global Change Biology 4:451–458.

Niklaus, P. A. 2007. Climate change effects on biogeochemical cycles, nutrients, and water supply. Pages 11–52 *in* P. C. D. Newton, R. A. Carran, G. R. Edwards, and P. A. Niklaus, editors. Agroecosystems in a changing climate. CRC Press, Boca Raton, Florida, USA.

Niklaus, P. A., J. Alphei, D. Ebersberger, C. Kampichler, E. Kandeler, and D. Tscherko. 2003. Six years of in situ CO_2 enrichment evoke changes in soil structure and biota of nutrient-poor grassland. Global Change Biology 9:585–600.

Niklaus, P. A., E. Glöckler, R. Siegwolf, and C. Körner. 2001*a*. Carbon allocation in calcareous grassland under elevated CO_2: a combined ^{13}C pulse-labelling/soil physical fractionation study. Functional Ecology 15:43–50.

Niklaus, P. A., E. Kandeler, P. W. Leadley, B. Schmid, D. Tscherko, and C. Körner. 2001*b*. A link between elevated CO_2, plant diversity and soil nitrate. Oecologia 127:540–548.

Niklaus, P. A., and C. Körner. 2004. Synthesis of a six-year study of calcareous grassland responses to in situ CO_2 enrichment. Ecological Monographs 74:491–511.

Niklaus, P. A., P. W. Leadley, B. Schmid, and C. Körner. 2001*c*. A long-term field study on biodiversity $\times$ elevated CO_2 interactions in grassland. Ecological Monographs 71:341–356.

Niklaus, P. A., P. W. Leadley, J. Stöcklin, and C. Körner. 1998*a*. Nutrient relations in calcareous grassland under elevated CO_2. Oecologia 116:67–75.

Niklaus, P. A., D. Spinnler, and C. Körner. 1998*b*. Soil moisture dynamics of calcareous grassland under elevated CO_2. Oecologia 117:201–208.

Niklaus, P. A., M. Wohlfender, R. Siegwolf, and C. Körner. 2001*d*. Effects of six years atmospheric CO_2 enrichment on plant, soil, and soil microbial c of a calcareous grassland. Plant and Soil 233:189–202.

Oades, J. M. 1984. Soil organic-matter and structural stability mechanisms and implications for management. Plant and Soil 76:319–337.

Oades, J. M. 1993. The role of biology in the formation, stabilization and degradation of soil structure. Geoderma 56: 377–400.

Oostenbrink, M. 1960. Estimating nematode populations by some selected methods. Pages 85–102 *in* J. Sasser and W. Jenkins, editors. Nematology. University of North Carolina Press, Chapel Hill, North Carolina, USA.

Owensby, C. E., J. M. Ham, A. K. Knapp, and L. M. Auen. 1999. Biomass production and species composition change in a tallgrass prairie ecosystem after long-term exposure to elevated atmospheric CO_2. Global Change Biology 5:497–506.

Pendall, E., A. R. Mosier, and J. A. Morgan. 2004. Rhizodeposition stimulated by elevated CO_2 in a semiarid grassland. 162:447–458.

Pimm, S. L., G. J. Russell, J. L. Gittleman, and T. M. Brooks. 1995. The future of biodiversity. Science 269:347–350.

Pulleman, M. M., J. Six, A. Uyl, J. C. Y. Marinissen, and A. G. Jongmans. 2005. Earthworms and management affect organic matter incorporation and microaggregate formation in agricultural soils. Applied Soil Ecology 29:1–15.

Reich, P. B., et al. 2001. Plant diversity enhances ecosystem responses to elevated CO_2 and nitrogen deposition. Nature 410:809–812.

Rillig, M. C., S. F. Wright, M. F. Allen, and C. B. Field. 1999. Rise in carbon dioxide changes soil structure. Nature 400: 628.

Salamon, J. A., M. Schaefer, J. Alphei, B. Schmid, and S. Scheu. 2004. Effects of plant diversity on collembola in an experimental grassland ecosystem. Oikos 106:51–60.

Schinner, F., R. Öhlinger, E. Kandeler, and R. Margesin. 1996. Methods in soil biology. Springer-Verlag, Berlin, Germany.

Schrader, S., and H. Q. Zhang. 1997. Earthworm casting: stabilization or destabilization of soil structure? Soil Biology and Biochemistry 29:469–475.

Southey, J. 1986. Laboratory methods for work with plant and soil nematodes. Volume 402 of Reference Book. Ministry of Agriculture, Fisheries and Food, London, UK.

Spehn, E. M., J. Joshi, B. Schmid, J. Alphei, and C. Körner. 2000. Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. Plant and Soil 224:217–230.

Spehn, E. M., et al. 2002. The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. Oikos 98:205–218.

Thomas, R. B., S. J. van Bloem, and W. H. Schlesinger. 2007. Climate change and symbiotic nitrogen fixation in agro-ecosystems. Pages 85–116 *in* P. C. D. Newton, R. A. Carran, G. R. Edwards, and P. A. Niklaus, editors. Agroecosystems in a changing climate. CRC Press, Boca Raton, Florida, USA.

Tilman, D., and J. A. Downing. 1994. Biodiversity and stability in grasslands. Nature 367:363–365.

Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 22:379.

Tisdall, J. M., and J. M. Oades. 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science 33: 141–163.

Vance, E. D., P. C. Brookes, and D. S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19:703–707.

van der Heijden, M. G. A., T. Boller, T. Wiemken, and I. R. Sanders. 1998. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology 79:2082–2091.

Viketoft, M., C. Palmborg, B. Sohlenius, K. Huss-Danell, and J. Bengtsson. 2005. Plant species effects on soil nematode communities in experimental grasslands. Applied Soil Ecology 30:90–103.

Walker, B. H. 1992. Biodiversity and ecological redundancy. Conservation Biology 6:18–23.

Wardle, D. A. 2002. Communities and ecosystems: linking the aboveground and belowground components. Princeton University Press, Princeton, New Jersey, USA.

Wardle, D. A., K. I. Bonner, G. M. Barker, G. W. Yeates, K. S. Nicholson, R. D. Bardgett, R. N. Watson, and A. Ghani. 1999. Plant removals in perennial grassland: Vegetation dynamics, decomposers, soil biodiversity, and ecosystem properties. Ecological Monographs 69:535–568.

Wardle, D. A., G. W. Yeates, W. M. Williamson, K. I. Bonner, and G. M. Barker. 2004. Linking aboveground and belowground communities: the indirect influence of aphid species identity and diversity on a three trophic level soil food web. Oikos 107:283–294.

Watt, M., W. K. Silk, and J. B. Passioura. 2006. Rates of root and organism growth, soil conditions, and temporal and spatial development of the rhizosphere. Annals of Botany 97: 839–855.

West, J. B., S. E. Hobbie, and P. B. Reich. 2006. Effects of plant species diversity, atmospheric [CO_2], and N addition on gross rates of inorganic N release from soil organic matter. Global Change Biology 12:1400–1408.

Wood, M., and A. M. McNeill. 1993. $^{15}N_2$ measurement of nitrogen fixation by legumes and actinorhizals: theory and practice. Plant and Soil 155–156:329–332.

Young, I. M., E. Blanchart, C. Chenu, M. Dangerfield, C. Fragoso, M. Grimaldi, J. Ingram, and L. J. Monrozier. 1998. The interaction of soil biota and soil structure under global change. Global Change Biology 4:703–712.

Zak, D. R., D. B. Ringelberg, K. S. Pregitzer, D. L. Randlett, D. C. White, and P. S. Curtis. 1996. Soil microbial communities beneath *Populus grandidentata* grown under elevated atmospheric CO_2. Ecological Applications 6:257–262.

Zaller, J. G., and J. A. Arnone. 1999*a*. Earthworm responses to plant species' loss and elevated CO_2 in calcareous grassland. Plant and Soil 208:1–8.

Zaller, J. G., and J. A. Arnone. 1999*b*. Interactions between plant species and earthworm casts in a calcareous grassland under elevated CO_2. Ecology 80:873–881.

Zanetti, S., U. A. Hartwig, A. Lüscher, T. Hebeisen, M. Frehner, B. U. Fischer, G. R. Hendrey, H. Blum, and J. Nösberger. 1996. Stimulation of symbiotic N_2 fixation in *Trifolium repens* L. under elevated atmospheric pCO_2 in a grassland ecosystem. Plant Physiology 112:575–583.

Zelles, L. 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology and Fertility of Soils 29:111–129.

APPENDIX

A table of microbial phospholipid fatty acids (PLFA), as functions of plant diversity and atmospheric CO_2 treatment (*Ecological Archives* E088-195-A1).